Metabolism of an insect diuretic hormone studied by on-line microbore liquid chromatography coupled to electrospray ionization mass spectrometry.

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The metabolism of peptide hormones by high affinity, specific endoproteinases is an essential part of hormone action. Usually this process is studied by incubating tissues with labeled peptide, and isolating fragments by reversed-phase liquid chromatography (RPLC). Fragments are identified by comparison to synthetic standards, or micro Edman degradation if enough is available.

We studied metabolism of the diuretic hormone of *Manduca sexta* (Mas-DH) incubated *in vitro* with Malpighian tubules, the target organ of Mas-DH. We identified its degradation products using microbore liquid chromatography coupled to ESI mass spectrometry. The high sensitivity of this technique allows identification of metabolites from Mas-DH added at 1 μM to incubations of a single tubule in 0.1 ml of medium. An accurate M_r value for a metabolite is usually sufficient for unambiguous identification. Mas-DH is cleaved by a number of proteases at 40 μM concentration, but only two proteases at 1 μM concentration.

A novel aspect of these studies was the formation of both possible mono-Met-sulfoxides and the bis-Met sulfoxide as major *in vitro* metabolites, especially at 1 μM. These oxidation products were less prominent at 40 μM. Their formation can be suppressed by addition of 1 mM Met to the medium, or by addition of catalase. A rational explanation for production of hydrogen peroxide by Malpighian tubules will be discussed.